Non-coding RNA

Genomes give rise to non-coding RNA molecules that regulate gene expression.

The expression of a non-coding RNA in plant roots.

The green fluorescence shows the expression of pMIR165a, a particular type of non-coding RNA called microRNA, that regulates the expression of a group of homeodomain transcription factors during development. However, the expression of pMIR165a is itself regulated similarly to other protein-coding genes. In this example, a gene called SCR-1 is required for the proper expression of pMIR165a (left) in plant root meristems. When plant roots with a nonfunctional mutant allele are examined (src-1, right), we see that pMIR165a is not expressed in its normal location. Subsequently, cells lacking pMIR165a lose proper transcriptional control of a group of homeodomain transcription factors during development that results in improper cellular differentiation in the plant root.


Topics Covered in this Module

- Non-coding RNA

Major Objectives of this Module

- Explain the significance of the discovery that non-coding RNA functions in gene expression.
- Explain how non-coding RNA molecules can affect gene regulation by changing chromatin structure.
- Explain how small non-coding RNA molecules can affect gene regulation by altering mRNA stability.
Astronomers think that most matter in the universe is "dark," meaning that it is made of matter we cannot observe and do not understand. In biology, small non-coding RNAs have been found in such abundance that they have been called the "dark matter" of the cell. Now, evidence is mounting that without these tiny RNA molecules, genes would not function properly. They regulate genes, modify chromatin structure, maintain genome integrity, and modulate mRNA stability. How did scientists overlook them for so long?

Non-coding RNA

One of the most surprising outcomes of the human genome project was the realization that the proportion of the human genome that codes for proteins is relatively small. Only 1.5% of our genome codes for specific protein products. The remaining DNA has not been shown to code for any specific protein products, and it is not used to make messenger RNA. What does all this other DNA do?

For decades, scientists assumed that most of this non-protein-coding DNA in eukaryotic genomes had no function and was not transcribed into RNA (Figure 1). Without having a functional protein end product, this part of the genome was considered "junk DNA." However, recent research has revealed that more than half, if not most, of non-coding DNA is transcribed, and that the resulting RNA actually has a function. **Non-coding RNA** (ncRNA) is RNA that is not translated into protein. Although ncRNA includes the familiar tRNA and rRNA that play important roles in transcription and translation, there are many other types of ncRNA that have recently been discovered and studied, many whose specific functions have yet to be defined.

**Figure 1: Non-coding DNA in a variety of organisms.**

All genomes contain non-coding DNA but in varying percentages. Generally, prokaryotes contain much less non-coding DNA as a proportion of their genome than eukaryotes.

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Non-coding RNAs function in gene regulation.
Scientists found the first clear evidence for ncRNAs in 1993 while studying gene regulation in the nematode *Caenorhabditis elegans* (Figure 2). They noticed that the abundance of protein made by one of the genes during development was regulated by a tiny piece of RNA, which they named a microRNA. A microRNA (miRNA) is a small, single-stranded RNA molecule with an average length of only 22 nucleotides. Originally thought to be unique to *C. elegans*, miRNAs were found years later in virtually all eukaryotic cells examined. What do these molecules do?

miRNA binds to transcribed complementary mRNA strands and functions by either blocking translation or targeting the mRNA for degradation. This action of miRNA is similar to another phenomenon uncovered in the 1990s by the American biologists Andrew Fire and Craig Mello. Fire and Mello were using an antisense RNA method to block the activity of certain developmental genes in *C. elegans*. Antisense RNA is a synthetic single-stranded RNA that is complementary to a targeted mRNA in a cell. When antisense RNA binds to mRNA, it silences the expression of the targeted mRNA. Fire and Mello found that mRNA blocking was more effective when double-stranded RNA (dsRNA) was introduced into the cell instead of single-stranded RNA. Somehow, the dsRNA blocked and completely silenced the mRNA, preventing translation. Fire and Mello called the phenomenon RNA interference (RNAi). The two scientists received the 2006 Nobel Prize in Physiology or Medicine for this work, which has since led to significant advances in the study of gene function.

Later research found that blocking translation did not result from dsRNAs binding to mRNA. Instead, it was discovered that dsRNAs are degraded into small (approximately 22-nucleotide long), single-stranded RNAs called small interfering RNAs (siRNAs), which bind to mRNA and block translation, just like miRNAs do. Both miRNA and siRNA are now considered RNAi agents; both are part of a system by which cells regulate genes post-transcriptionally in the cytoplasm. What structural features allow these small RNAs to silence genes? Scientists found that both use similar mechanisms and that both are the product of double-stranded RNA degradation.
Figure 3 shows the synthesis of an miRNA molecule. The gene for the miRNA is transcribed as a single RNA molecule with many self-complementary regions. When these regions base pair with each other, the RNA molecule folds back on itself and forms a number of small, double-stranded hairpin loops. The RNA molecule is cleaved into shorter double-stranded segments by an enzyme complex known as the Microprocessor complex. Each segment then leaves the nucleus and enters the cytoplasm, where another enzyme called Dicer cuts the segments into short, 20-base-pair fragments — the mature miRNAs. Next, the miRNA is transferred to the Argonaute (AGO) enzyme complex, which degrades one of the strands of the double-stranded miRNA, leaving a single strand of miRNA associated with the enzyme. The combination of the AGO complex and the miRNA is known as the RNA-induced silencing complex, or RISC. Using the sequence of the miRNA, the RISC identifies, targets, and binds to complementary mRNA strands for cellular degradation or silencing. siRNAs are associated with the same protein complexes, and are produced with the help of the same Dicer enzymes. siRNAs differ in that they are produced from longer, linear dsRNA molecules.
Figure 3: The synthesis and function of miRNA.
In the first step, the miRNA precursor is transcribed from its gene within the nucleus. Before the miRNA precursor leaves the nucleus, the 5' cap and poly(A) tail of the precursor are removed, and the precursor is cleaved into smaller segments by the Microprocessor complex. In the cytoplasm, the Dicer enzyme removes hairpin loops from the segments.
and processes the segments into short, 20-base-pair fragments of double-stranded miRNA. Next, the AGO protein complex binds to the dsRNA, triggering Dicer to detach, and digests one of the two miRNA strands, leaving one strand of miRNA bound to the AGO protein complex and forming the RNA-induced silencing complex (RISC). The RISC can then base-pair with complementary nucleotides to identify its target mRNA sequence. If the target sequence is found, the RISC removes the mRNA's 5' cap and poly(A) tail. Without the cap and tail, the mRNA molecule becomes targeted for degradation.

Test Yourself

How do miRNAs and siRNAs act post-transcriptionally to silence genes?

Submit

Future perspectives and open questions.
The role of miRNA and siRNA as post-transcriptional gene regulators has been known for over a decade. Other types of ncRNA were discovered more recently and are less well-characterized. One ubiquitous group is that of the long, non-coding RNAs (lncRNAs). These ncRNAs differ from miRNA and siRNA in length. Most lncRNAs are many hundreds of nucleotides long. Scientists identified them while searching genomes for protein-coding regions. They came upon long, transcribed areas of RNA without open reading frames and other necessary translation signals. Many researchers believed that these lncRNAs were byproducts of protein-producing translation activities.

Scientists soon learned that some lncRNAs play important roles in cell differentiation and development by influencing how genes are expressed in different cell lines. Changing patterns of lncRNA expression can have an effect on the developmental path a cell takes. These long RNA pieces can also serve as a scaffold for proteins necessary for specific functions, such as chromatin remodeling. lncRNA might activate these specific proteins by binding to them.

Scientists are exploring other roles that lncRNAs play in cells. Some lncRNAs appear to have multiple roles in gene expression; some may have no function at all. In 2010, scientists made the surprising discovery in Drosophila that an lncRNA containing a short open reading frame actually codes for a functional peptide, even though the lncRNA is not considered a traditional mRNA. This peptide plays an important role in regulating genes coding for Drosophila bristle development. This was the first example of an lncRNA — indeed any ncRNA — found to make a peptide product. The peptide was small, containing fewer than 30 amino acids, and because of its small size, it was difficult to detect. Scientists were puzzled by this discovery. Is this lncRNA part of an entirely new class of ncRNAs? Are there other ncRNAs that code for peptides? How many of these peptides, as one scientist wrote, "hide in plain sight?"

Non-coding RNAs can change chromatin structure and disarm transposons.
Preventing translation is an important function that siRNAs and miRNAs provide as RNAi agents. Some siRNAs also bind to a set of specialized protein complexes that promote the formation of heterochromatin. Recall that heterochromatin is tightly packed, transcriptionally silent DNA that may contain relatively few genes. Usually, heterochromatin is enriched in mobile
siRNA can be used by the cell to identify chromosomal regions containing transposons and to condense those regions into heterochromatin to prevent the transposons from being transcribed. By binding to heterochromatin protein complexes, siRNA helps identify the area of the chromosome to be targeted for heterochromatin formation. In doing so, the siRNAs inactivate the target region by preventing transcription of genes on that portion of the chromosome, including any transposons within it (Figure 4).

Another type of ncRNA molecule, called piwi-interacting RNA (piRNA), appears to act as a sentinel to directly target transposons in germ cells. When a new transposon invades a cell, complementary piRNAs, carried within the Argonaute-like Piwi protein, bind to the transposon product, effectively silencing it. By targeting transposons, the piRNAs prevent these destructive elements from interfering with germ cell formation, helping to maintain germline integrity. In mammals, piRNAs are found only in male germ cells and, in some cases (including in humans), they are required for spermatogenesis.

Although piRNAs are currently the largest class of ncRNAs expressed in animal cells, they are just beginning to be fully characterized. They are a bit larger than miRNA and siRNA but only by a few nucleotides. The pathways that synthesize piRNA are distinct from those that process miRNA and siRNA.
A different kind of ncRNA is involved in the silencing of the extra X chromosome in female mammalian cells. Recall that female cells contain two X chromosomes (male cells contain only one). An IncRNA called Xist coats one of the X chromosomes and functions along with other factors to package it into heterochromatin, thereby inactivating it (Figure 5). Many scientists see the Xist inactivation process as a model system for developmental epigenetics. In fact, ncRNAs appear to play critical roles in many epigenetic processes in cells. For example, in *C. elegans*, researchers have found that a single incidence of RNAi in heterochromatin induces gene silencing that is inherited over many generations.

**Figure 5: X chromosome inactivation by Xist.**
An ncRNA called Xist (pink) coats one of the two X chromosomes in this nucleus from a female mouse cell, helping to compact it into heterochromatin and inactivating it. © 2011 Nature Publishing Group


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**Enhancer sequences produce eRNAs.**
Not all ncRNAs are gene silencers. Some are gene enhancers. For example,
**enhancer RNA (eRNA)** is made by enhancer regions in genes and appears to amplify protein production when the genes associated with the enhancers are expressed. Scientists have long known that enhancer regions bind with transcription factors to enhance the transcription of targeted genes by interacting with protein complexes at transcription start sites. Enhancers work from a distance — they are sometimes located in regions of DNA far upstream or downstream from transcription sites and can even be on a different chromosome. New research points out that many of these enhancer sites are transcribed into eRNAs.

eRNA was discovered in mouse neuron cells. When researchers artificially stimulated a mouse neuron gene, the gene's enhancer region upstream of the gene produced eRNA. The production of eRNA correlated with an increase in the gene's protein response. The mouse genome contains thousands of enhancers and, presumably, thousands of eRNAs. While it is not yet clear exactly how eRNAs work or even whether all of them are indeed functional, some scientists have suggested that in neurons they might be critical in developing new synaptic connections and in this regard might play a role in learning and memory retention.

**Future perspectives and open questions.**

It is not yet clear how widespread eRNAs are in the human brain, but scientists have found many other ncRNAs that some speculate might have played a role in the evolution of the human brain. How? Although humans have approximately the same number of genes as many other organisms, including nematodes and fruit flies, humans have many more ncRNAs. Could it be possible that multilayered regulation of genes by these ncRNAs in the human brain explains the brain's complexity? And could ncRNAs that regulate other kinds of genes also contribute to the uniqueness of humans? Intriguingly, scientists have found that differences in IncRNA sequences between the human and chimpanzee (*Pan troglodytes*) genomes account for nearly half of the genetic variation separating these two species.

**Non-coding RNAs have potential medical uses.**

ncRNAs are ubiquitous in the human body, so it is not surprising that they are associated with many diseases, including some cancers and neurodegenerative diseases. There is evidence, for example, that some ncRNAs play a role in Alzheimer's disease. In 2011, a group in Italy found that brain inflammation triggers the transcription of ncRNAs that alter a protein, which then interferes with nerve conductance and promotes production of amyloid-β, the main protein constituent of plaques in the brains of patients with Alzheimer's disease.

If ncRNAs are associated with some diseases, could they be used to treat those diseases? The more scientists understand ncRNAs' function in both health and disease, the better equipped they will be to develop ncRNA-specific treatments. Already, there has been some progress in using RNA interference in therapy for eye diseases such as age-related macular degeneration (AMD). In the future, RNAi therapy might be useful in silencing some cancer genes.

Many scientists think that the RNAi mechanism in cells functions to defend against viruses, since it relies on dsRNA. dsRNA molecules are rare outside of viruses. This function of RNAi has been identified in plants: when a virus enters a plant cell, the cell activates RNAi pathways to destroy it. Similar responses have been shown to occur in some animal cells. RNAi is therefore a promising tool in the development of anti-viral therapies. It might be possible, for instance, to synthesize short, single-stranded siRNA mimics and insert them into human cells to target specific viruses.

Despite promising investigations into the use of ncRNAs to develop medical therapies, many questions remain. How many ncRNAs are there? Why are
there so many different types? Do they serve multiple roles or are they specialized? How many make peptides — and why? While a handful of ncRNAs have been characterized and their pathways explored, there are thousands more that, like dark matter, remain hidden. It is known that most of the small RNA molecules that have been identified play important roles in gene regulation and genome stability. Scientists estimate that over half of all human genes are regulated by miRNAs. As we learn more about ncRNAs, we may find that they serve as important a role in cellular differentiation and embryonic development as protein molecules do in the regulation of cell function.

IN THIS MODULE
- Non-coding RNA
- Summary
- Test Your Knowledge

WHY DOES THIS TOPIC MATTER?
- Cancer: What's Old Is New Again
  Is cancer ancient, or is it largely a product of modern times? Can cutting-edge research lead to prevention and treatment strategies that could make cancer obsolete?
- Stem Cells
  Stem cells are powerful tools in biology and medicine. What can scientists do with these cells and their incredible potential?
- Synthetic Biology: Making Life from Bits and Pieces
  Scientists are combining biology and engineering to change the world.

PRIMARY LITERATURE
- Interfering with microRNAs to control gene expression
  Silencing of microRNA families by seed-targeting tiny LNAs.
  View | Download
- Can we expand the genetic code?
  Converting nonsense codons into sense codons by targeted pseudouridylation.
  View | Download
Summary

OBJECTIVE Explain the significance of the discovery that non-coding RNA functions in gene expression.

The recent discovery that ncRNAs are involved in regulating gene expression changed the way scientists study and think about the genome. ncRNAs regulate the expression of many genes and may play important roles in embryonic development, cellular differentiation and genome stability.

OBJECTIVE Explain how small non-coding RNA molecules can affect gene regulation by altering mRNA stability.

Small ncRNA molecules, like siRNAs and miRNAs, modulate gene expression by binding to mRNAs in the cytoplasm. When mRNAs are targeted by siRNAs or miRNAs, their translation is blocked, or they are degraded. These molecules are part of a process called RNA interference.

OBJECTIVE Explain how non-coding RNA molecules can affect gene regulation by changing chromatin structure.

Some ncRNA molecules, such as lncRNA and piRNA, affect gene expression by altering chromatin structure. These molecules bind to protein complexes that work together to form heterochromatin, and to target areas of the genome from which they were transcribed. Heterochromatin formation reversibly inactivates genes and transposable elements by making them unavailable for transcription.

Key Terms

**enhancer RNA (eRNA)** Enhances protein production when gene is turned on.

**microRNA (miRNA)** Small, single-stranded RNA molecule.

**non-coding RNA** RNA that is not translated into a protein.

**piwi-interacting RNA (piRNA)** Target transposons, preventing them from interfering with germ cell production.

**RNA interference (RNAi)** Blocking and silencing of the mRNA to prevent translation.

**small interfering RNA (SiRNA)** A type of noncoding RNA that binds to mRNA to prevent translation.
Incredible potential?

Synthetic Biology: Making Life from Bits and Pieces

Scientists are combining biology and engineering to change the world.

PRIMARY LITERATURE

Interfering with microRNAs to control gene expression
Silencing of microRNA families by seed-targeting tiny LNAs.
View | Download

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View | Download
Principles of Biology

53 Non-coding RNA

Test Your Knowledge

1. How do microRNAs (miRNAs) regulate genes?
   - miRNAs do not affect translation or transcription.
   - Ribosomes bind to miRNAs and translate protein.
   - miRNAs bind to DNA and transcribe mRNA.
   - miRNAs bind to mRNA and prevent translation.
   - miRNA and mRNA perform the same functions.

2. What specific role does small interfering RNA (siRNA) have in the formation of heterochromatin?
   - siRNA binds to ribosomes and guides ribosomes to targeted gene sequences.
   - siRNA does not affect heterochromatin formation.
   - siRNA binds to enzyme complexes and guides them to targeted DNA.
   - siRNA binds to microRNA (miRNA), forming heterochromatin.
   - siRNA forms heterochromatin by binding to DNA directly.

3. Which type of non-coding RNA (ncRNA) is the basis of the RNA interference (RNAi) technique?
   - mRNA
   - microRNA (miRNA)
   - piwi-interacting RNA (piRNA)
   - long non-coding RNA (IncRNA)
   - tRNA

4. Why is it important for scientists to understand how non-coding RNA (ncRNA) functions in gene expression?
   - ncRNA may alter the production of important proteins.
   - ncRNA is an important intermediate in DNA replication.
   - Without ncRNA, ribosomes cannot read mRNA and make proteins.
   - Some enzyme complexes require ncRNA to perform cellular respiration.
   - ncRNA is unrelated to mRNA and protein synthesis.

5. How does piwi-interacting RNA (piRNA) interact with transposons?
   - piRNA associates with small interfering RNA (siRNA) and binds to ribosomes.
   - piRNA transcribes transposons into mRNA.
   - piRNA is translated into anti-transposon proteins.
   - piRNA targets transposons for inactivation.
   - piRNA has nothing to do with transposons in the genome.

6. How does the discovery of the role of non-coding RNA (ncRNA) in gene expression affect how scientists study gene expression?
   - ncRNA takes the place of mRNA as the most important DNA-to-protein intermediate.
   - ncRNA is a more stable molecule than DNA.
   - Scientists must consider the effect ncRNA has on protein production.
   - Scientists do not think ncRNA is important because it does not affect gene expression.
It is important to recognize that ncRNA codes for proteins in addition to mRNA.